PROC (BAYL UNIV MED CENT)
2022;35(1):84–85

https://doi.org/10.1080/08998280.2021.1984792

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Hemoglobin Wayne causing a falsely elevated hemoglobin A1c

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ABSTRACT

Hemoglobin A1c (HbA1c) is an important tool for diagnosis and management of patients with diabetes mellitus (DM). However, hemoglobin variants can interfere with laboratory assays and lead to inaccurate results. This study describes a patient who was found to have falsely elevated HbA1c values secondary to hemoglobin Wayne, a rare hemoglobin variant. Although hemoglobin Wayne is often clinically silent, falsely elevated HbA1c results could lead to unnecessary medical interventions that could cause patient harm. This variation in results highlights the importance of utilizing additional measurements such as glucose readings and evaluating for hemoglobin variants when results are discordant.

KEYWORDS Diabetes mellitus; hemoglobin A1c; hemoglobin electrophoresis; hemoglobin Wayne; high-performance liquid chromatography

Hemoglobin A1c (HbA1c), or glycated hemoglobin, is one of the standard biomarkers for glycemic control in patients with diabetes mellitus (DM) and is recognized in the diagnostic criteria of the American Diabetes Association.[1,2] HbA1c testing is subject to interference by a variety of conditions including hemoglobin variants. Examples of more common and easily detectable hemoglobin variants include hemoglobin S, C, D, and E. Ion-exchange high-performance liquid chromatography (HPLC) methods are specifically prone to interference from amino acid substitutions that cause changes in the net charge of hemoglobin.[3–6] Hemoglobin Wayne, a rare hemoglobin variant first described in a Caucasian neonate born in Pike County, Georgia, in 1976, has been described in previous case studies to falsely elevate HbA1c when measured with specific HPLC assays.[7–9] The variant is the result of a frame-shift mutation and leads to an elongated alpha chain. The two forms, hemoglobin Wayne Asn and hemoglobin Wayne Asp, are characterized by asparagine or aspartic acid located at residue 139.[10] Although hemoglobin Wayne is often clinically silent, the impact it has on HbA1c values makes consideration of this variant important in clinical practice.[6,9,11]

CASE DESCRIPTION

A 50-year-old Hispanic woman with hypothyroidism, hyperlipidemia, and hypertension was referred to the endocrinology clinic for management of type 2 DM. She was initially diagnosed with type 2 DM by her primary care provider based on an HbA1c reading of 10.3% that was obtained as part of an annual physical exam. Fasting glucose readings at that time were within normal range, from 84 to 91. Home random glucose readings ranged from 75 to 120 mg/dL and postprandial readings were up to the 140 s, with rare occurrences of readings of 200 to 220 mg/dL after heavy meals. Her body mass index was 28.8 kg/m^2. Lifestyle changes were recommended and she adjusted her diet and began running 5K races as training for a half-marathon. During her initial encounter with an endocrinologist, she denied acute symptoms including blurry vision, polydipsia, polyphagia, weight loss, palpitations, abdominal discomfort, nausea, emesis, constipation, diarrhea, lightheadedness, confusion, diaphoresis, or leg numbness. She was started on metformin 500 mg twice daily and instructed to attend diabetes education classes and to check blood sugars before meals and at bedtime.

Repeat laboratory determinations approximately 4 months after the initial ones demonstrated fasting glucose of 91 mg/dL and HbA1c of 10.4% with the Bio-Rad Variant II system, which was the same platform that was previously used. Urine

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The authors report no conflicts of interest. The patient has given permission for the publication of this case.
Received July 30, 2021; Revised September 12, 2021; Accepted September 16, 2021.

Table 1. Hemoglobin electrophoresis cascade results

Hemoglobin type	Patient value (%)	Reference value (%)
A1	85.4	95.8–98.0
A2	1.7	2.0–3.3
F	0.2	0.0–0.9
Hemoglobin Wayne I	7.3	0
Hemoglobin Wayne II	5.4	0

microalbumin was below the assay limit. Complete blood count and comprehensive metabolic panel results were within normal limits. Fasting blood glucose readings ranged from 80 to 95 mg/dL, which prompted additional evaluation.

Further review of HbA1c throughout the previous 3 years using immunoassay with the Bio-Rad Variant II displayed results of 9.7% to 10.6%, with no significant change when lifestyle changes or metformin were added. Point-of-care testing with Siemens digitally controlled amplifier was completed within the endocrinology clinic with results of 5% to 6%. Fructosamine testing via ARUP Laboratories in Salt Lake City, Utah, was within normal range at 233 to 251 µmol/L. No hemoglobin interference was noted on chromatogram with the Bio-Rad Variant II system. Hemoglobin electrophoresis cascade and mass spectrometry completed through Mayo Clinical Laboratories confirmed the presence of the hemoglobin Wayne variant *(Table 1)*.

DISCUSSION

This case demonstrates the importance of evaluating for hemoglobinopathies in the setting of discordant lab values. HbA1c creation occurs through glycosylation, the nonenzymatic attachment of glucose to the N-terminal valine within the beta globulin chain.[12] This result is an important biomarker used for diagnosis and management of DM; therefore, interference in this modality is important to identify. This patient presented with elevated HbA1c findings on one HbA1c platform, which contradicted normal results from HbA1c point-of-care testing, fructosamine, and fasting glucose readings.

In this case, hemoglobin Wayne led to inappropriate elevation in HbA1c when evaluated using the Bio-Rad Variant II ion-exchange HPLC method. The interaction of hemoglobin Wayne with this method of testing has been described in previous cases.[7–9] This method is thought to be affected because hemoglobin Wayne has an amino acid substitution that confers a similar charge to A1c, leading to similar retention time within the platform and an overestimation of A1c concentration. Similarly charged fractions may not be separated within these peaks, which is especially true of older analyzers that have not been adjusted for rare variants. For patients with hemoglobin Wayne, alternative methods of HbA1c testing such as immunoassay and boronate affinity chromatography may be used for accurate readings since they do not rely on net charges of the components it separates.[13]

In summary, rare hemoglobin variants including hemoglobin Wayne may present as discordant lab results without reported symptoms or other laboratory abnormalities. Specific variants may also be undetectable with some testing platforms such as those that rely on ion exchange. This study serves as an example of the importance of recognition of discordant results within a clinical scenario to provide optimal patient care and ensure patient safety.

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